

evidently increased, suggesting the acidification between the membrane-surface space. Additionally, the degree of the acidification was proportional to the concentration of uncaged ATP ranging from 0.2 μM to 0.7 μM . These results suggest that we successfully detected the proton pumping activity sensitively and quantitatively. As many kinds of Caged compounds and ion sensitive fluorescent dyes are commercially available, this technique can be applied to other ion pumps and transporters.

Mitochondrial & Chloroplast Transport

1539-Pos Board B431

Ran GTPase-Independent and Stereochemical Control of Kinesin-1 and Mitochondrial Motility by Domains of Ran-Binding Protein-2

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The microtubule-based motor proteins, dynein and kinesin-1, mediate fast mitochondrial trafficking, but the mechanisms underlying the regulation of mitochondrial motility are ill-defined. The Ran-binding protein 2 (RanBP2) is a pleiotropic and multimodular protein, which couples directly with the kinesin-1 isoforms, KIF5B/KIF5C, via its tripartite domains, the kinesin-binding domain (KBD) and the Ran GTPase-binding domains, RBD₂ and RBD₃. The coupling of RBD₂-KBD-RBD₃ to kinesin-1 activates its ATPase activity ~30-fold and with activation kinetics that is biphasic and cooperative. Here, we employ structure-function, biochemical, kinetic and cell-based assays with time-lapse live-cell microscopy of over 260,000 mitochondrial motility-related events to probe the interplay between Ran GTPase and RBD₂-KBD-RBD₃ on kinesin-1 activation and mitochondrial motility. We uncover mutually exclusive subdomains in RBDs toward Ran GTPase binding, kinesin-1 activation and modulation of mitochondria motility. The RBDs exhibit Ran-GTP-independent, subdomain and stereochemical-dependent discrimination on the biphasic activation kinetics of kinesin-1 or regulation of mitochondrial motility. Remarkably, RBD₂-KBD-RBD₃ and KBD alone exert opposing effects on the equilibrium between the stationary and motile phases of mitochondria and multiple biophysical parameters of mitochondrial motility. Further, the regulation of the bidirectional transport of mitochondria by either RBD₂-KBD-RBD₃ or KBD is highly coordinated, since their effects are accompanied always by changes in motile biophysical parameters of opposite-polarity. These studies uncover Ran GTPase-independent antagonizing and multimodal mechanisms of kinesin-1 activation and regulation of mitochondrial motility by distinct domains of RanBP2. Further, they open new venues toward the pharmacological harnessing of mechanisms regulating kinesins, mitochondrial motility or RanBP2 in a variety of disparate disorders.

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MICU1 is an Essential Gatekeeper for MCU-Mediated Mitochondrial Ca²⁺ Uptake that Regulates Cell Survival

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Mitochondrial Ca²⁺ uptake is mediated by an inner membrane Ca²⁺ channel called the uniporter. Ca²⁺ uptake is driven by the considerable voltage present across the inner membrane ($\Delta\Psi_m$) generated by proton pumping by the respiratory chain. Mitochondrial matrix Ca²⁺ concentration ($[\text{Ca}^{2+}]_m$) is maintained 5-6 orders of magnitude lower than its equilibrium level, but the molecular mechanisms for how this is achieved are not clear. Here we demonstrate that the mitochondrial protein MICU1 is required to preserve normal $[\text{Ca}^{2+}]_m$ under basal conditions. In its absence, mitochondria become constitutively loaded with Ca²⁺, triggering excessive reactive oxygen species generation and sensitivity to apoptotic stress. MICU1 interacts with the uniporter pore-forming subunit MCU and sets a Ca²⁺ threshold for mitochondrial Ca²⁺ uptake without affecting the kinetic properties of MCU-mediated Ca²⁺ uptake. Thus, MICU1 is a gatekeeper of MCU-mediated mitochondrial Ca²⁺ uptake that is essential to prevent mitochondrial Ca²⁺ overload and associated stress.

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Translocation of Knotted Proteins into Mitochondria

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In recent years a surge of interest has arisen in properties and function of knotted proteins. As more and more knotted structures are discovered in the

Protein Data Bank, it becomes increasingly important to understand how, if at all, the non-trivial topology affects the protein's function in the cell. In particular, it has been hypothesized that the presence of a knot in the polypeptide backbone may affect the ability of knotted proteins to be degraded in proteasome or translocated through the intercellular membranes, e.g. during import into mitochondria. In these processes, the translocating proteins typically have to pass through constrictions that are too narrow to accommodate folded structures, thus translocation must be coupled to protein unfolding. However, as shown in a number of theoretical and experimental studies the protein knots get tightened under the tension. The radius of gyration of the tight knot is about 7-8 Angstrom, whereas the diameters of the narrowest constriction of the mitochondrial pores are in the 12-15 Angstrom range, making it possible for the knots to get stuck during the translocation process. In this communication, we report the result of molecular dynamics simulations of knotted protein translocation which show how such topological traps might be prevented by using a pulling protocol of a repetitive, on-off character. Such a repetitive pulling is biologically relevant, since the mitochondrial import motor, like other ATPases transform chemical energy into directed motions via nucleotide-hydrolysis-mediated conformational changes, which are cyclic in character. This research has been supported by the Polish NCN grant N N202 055440.

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On the Role of Positively Charged Residues of TM2 Domain in the Chloride Transport of Human UCP2

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Located in the inner mitochondrial membrane, uncoupling proteins (UCPs) dissipate the proton electrochemical gradient causing reduction in the rate of ATP synthesis. Among five human UCP homologues, UCP2 is unique with its ubiquitous expression in various tissues. This important feature has been attributed to UCP2's multiple physiological roles in tissues, including its involvement in protective mechanisms against oxidative stress, glucose and lipid metabolisms. Despite numerous physiological studies, UCP2 function in cell remains poorly understood. UCP2 proton transport is regulated by purine nucleotides such as ATP, ADP, GTP and GDP. In addition, UCP2 has also been observed to transport chlorides and other small anions. Identification of the key amino acid residues in UCP2 in proton, anion transport and regulation will help determine the protein's mechanism of action in cells. It has been established that positively charged residues on transmembrane helix 2 (TM2) of UCP1 and UCP2 are crucial for chloride transport. However, a full understanding of the transport mechanism is yet to be achieved. More importantly, some of these residues are also involved in the UCP2 proton transport regulation. To further understand the ion transport of UCP2, four TM2 mutants have been made (R76Q, R88Q, R96Q, and K104Q). Over-expressed proteins were purified and reconstituted into liposomes for structural and functional studies. All mutants share an overall helical conformation with wtUCP2. Using anion-sensitive fluorescent probes, proton and chloride transport of UCP2 mutants are examined to determine the effect of each mutation on the ion transport of UCP2. In addition, Mant-modified purine nucleotide will be used to study the binding of UCP2 and its mutants to purine nucleotides. Overall, the outcome of this study will provide a more detailed molecular image of UCP2 ion transport mechanism.

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Regulation of Mitochondrial Ca²⁺ Dynamics by Inorganic Phosphate

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The mitochondrial Ca²⁺ uniporter (mCU) is the main influx pathway for the uptake of mitochondria Ca²⁺ and plays an important role in regulating energy production as well as cell life and death. In our previous study, we reported two modes of the mCU (mode1 and mode 2) that have different properties with respect to Ca²⁺ affinity (mode1 > mode 2) and Ru360 sensitivity (mode 1 < mode 2). This study further investigates the role of concomitant inorganic phosphate (Pi) transport on mitochondrial uptake of Ca²⁺ mediated by the mCU in isolated cardiac mitochondria loaded with mitochondrial and extra-mitochondrial Ca²⁺ fluorescent dyes (Fura-FF and Calcium Green-5N). In Pi-depleted mitochondria, the maximal Ca²⁺ uptake rate is shown here to be limited by extramitochondrial Pi. The mitochondrial Ca²⁺ uptake rate was accelerated in a concentration-dependent manner by phosphate concentrations ranging from 0.01 mM to a maximum at ~0.1 mM. The effect of Pi on mode 2